THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 248, No. 14, Issue of July 25, pp. 5144-5159, 1973
Printed in U.S.A.

Pyridine Nucleotide Metabolism in *Escherichia coli*

III. BIOSYNTHESIS FROM ALTERNATIVE PRECURSORS *IN VIVO**

(Received for publication, November 8, 1972)

JOHN MCLAREN‡

From the Division of Biology, Kansas State University, Manhattan, Kansas 66502

DOMINGO T. C. NGO§ AND BALDOMERO M. OLIVERA

From the Department of Biology, University of Utah, Salt Lake City, Utah 84112, and the Department of Biochemistry, University of the Philippines, Manila, Philippines

SUMMARY

In *Escherichia coli*, the nicotinamide moiety of pyridine nucleotides may be derived from *de novo* synthesis of the pyridine ring or through the uptake of either nicotinic acid or nicotinamide. *De novo* synthesis is the least preferred pathway; it is suppressed if an exogenous source is available. Nicotinamide is kinetically preferred over nicotinic acid; the data are consistent with the hypothesis that nicotinamide can pass through the bacterial membrane much faster than can the charged nicotinic acid, and once inside the cell, the amide is efficiently converted to the acid. Thus even at low concentrations nicotinamide is instantly taken up by cells, but at concentrations of less than 8×10^{-6} M nicotinic acid in the medium, the rate of entry of the nicotinic acid into the cell appears to be the rate-limiting step in the synthesis of pyridine nucleotide from exogenous niacin.

Under conditions where sufficient exogenous niacin or nicotinamide is present so that entry into the cell is not limiting, the rate of pyridine nucleotide biosynthesis is apparently regulated by the rate of conversion of nicotinic acid → nicotinic acid mononucleotide, as suggested by IMSANDE and PARDEE ((1962) *J. Biol. Chem.* 237, 1305). Since the rate of conversion of nicotinamide → nicotinic acid is not regulated, excess nicotinic acid that is formed from nicotinamide is continually excreted into the medium.

The nicotinamide moiety of the pyridine nucleotides may come from several alternative precursors in the bacterium *Escherichia coli*: by endogenous synthesis from aspartate and glycerol, or through uptake of exogenous nicotinic acid or nicotinamide (2). In this paper we describe studies which define how the cell uses these alternative precursors so that it maintains fairly constant levels of intracellular pyridine nucleotide under different growth conditions.

A number of *in vitro* studies which suggest possible control points for pyridine nucleotide biosynthesis in *E. coli* have been recorded in the literature. Nicotinic acid mononucleotide (N_aNM) pyrophosphorylase (3), as well as a cell-free system for the *de novo* synthesis of niacin from aspartate and glycerol (4), have been reported to be repressible. It is an aim of this paper to define how such potential control mechanisms may actually operate in the intact cell. Our interest in these studies also stems from the fact that our previous studies on pyridine nucleotide metabolism (5, 6) have always utilized exogenous niacin as the source of the nicotinamide moiety. We seek to measure how much pyridine nucleotide metabolism is perturbed by the presence of substantial levels of exogenous precursors in the medium.

Finally, it has been postulated that intestinal microorganisms play a role in the deamidation of nicotinamide to nicotinic acid in animals (2, 7). We have observed that nicotinamide is in fact converted to extracellular nicotinic acid by the bacterium *E. coli*. The mechanism by which this occurs is clarified by the present studies.

EXPERIMENTAL PROCEDURES

Materials—[^{14}C]- and [^{3}H]nicotinic acid were purchased from Amersham-Searle Corp. and purified as described previously (2). [^{14}C]Nicotinamide was obtained from New England Nuclear Corp.

DEAE-Sephadex and alcohol dehydrogenase were purchased from Sigma. All other materials were described in the preceding papers of this series (2, 3).

Bacterial Strains and Media—The strain used in most experiments was *E. coli* 15T$^-$ (strain 555-7). Two other strains, *E. coli* 15T$^-$ nic$^-$ (4) and RS126 (a nic C$^-$, nicotinamide deamidase$^-$ strain) (8) were used for some experiments. We are indebted to Dr. R. K. Gholson, Oklahoma State University, for the gift of the latter strain.

The growth media for these bacteria have been described previously (5).

* This investigation was supported by Grants GB8610 and GB22869X from the National Science Foundation, and by a grant from Natural Science Research Center, University of the Philippines. A preliminary account of some of this work has been presented elsewhere (1).

‡ Supported by a Genetics Training Grant Fellowship from the National Institutes of Health.

§ Supported by a Summer Student Research Fellowship of the China Medical Board.

Determination of Specific Activity of Niacin and Nicotinamide—The concentration of the niacin and nicotinamide stock solutions was determined by making appropriate dilutions and measuring absorbance at several wave lengths using a Zeiss PMQ II spectrophotometer. The absorbance profile agreed with published spectra (9) with significant deviation only at extreme ends of the absorption peak. The niacin concentration was calculated using a molar absorption coefficient of 4200 for niacin and 2780 for nicotinamide at 262 nm (9). To determine the radioactivity of the niacin and nicotinamide, aliquots from the solutions used to measure optical density were spotted on Millipore filters, dried, and counted in a Beckman liquid scintillation counter.

Determination of Specific Activity of Intracellular DPN—A culture of *E. coli* 15T$^-$ was grown in an M9 salts medium as described previously except that various concentrations of [^{14}C]-niacin were present in the medium. The niacin concentrations used were: 4.06×10^{-7} M (0.05 μg per ml), specific activity, 2.33×10^{10} cpm per mmole; 8.125×10^{-7} M (0.1 μg per ml), specific activity, 1.16×10^{10} cpm per mmole; 1.625×10^{-6} M (0.2 μg per ml), specific activity, 1.16×10^{10} cpm per mmole; 4.06×10^{-6} M (0.5 μg per ml), specific activity, 4.65×10^{9} cpm per mmole; and 8.125×10^{-6} M (1 μg per ml), specific activity, 4.65×10^{9} cpm per mmole. During the period of exponential growth samples were taken for Coulter counting and for radioactivity measurement. When the culture had reached a density of 6 to 7×10^{8} cells per ml, the cells were collected by centrifugation in the GSA rotor of the Sorvall centrifuge at 6000 rpm ($5860 \times g$) for 10 min. The pellets were resuspended in M9 salts medium containing 40 μg per ml of [^{12}C]niacin and recentrifuged in the GSA rotor and Sorvall centrifuge at 6000 rpm for 10 min. The pellet was resuspended in about 5 ml of 0.01 M Tris, 0.005 M β-mercaptoethanol, 0.005 M EDTA, pH 8.3, and was sonicated in a Raytheon sonicator for 10 to 15 min. The solution was then acidified to approximately pH 2 with 0.33 N HCl and centrifuged in the SS 34 rotor of the Sorvall centrifuge at 7K ($5900 \times g$) for 10 min. The supernatant was kept at 0°, then neutralized to pH 6.5 to 7.5 with ice-cold 1 M NaOH.

A column of DEAE-Sephadex was prepared by washing the DEAE-Sephadex with 0.01 M Tris, pH 8.0, and pipetting the slurry into a column, 12-mm inside diameter, to a height of 5 to 6 cm. The column was washed several times with 0.01 M Tris, pH 8, and the cell extract was applied. A stepwise elution was carried out using 15 ml of 0.02 M Tris, pH 8, 15 ml of 0.03 M Tris, and 50 ml of 0.04 M Tris. The absorbance at 260 nm was directly recorded on chart paper using a Gilford model 2400 spectrophotometer with a flow cell attachment. The eluted material was collected in 5-ml fractions and aliquots were taken from each fraction to locate the radioactivity peaks. DPN was routinely eluted by the 0.04 M Tris; at least 65% of the absorbance at 260 nm in 0.04 M Tris eluate is due to DPN. The DPN peak was pooled and concentrated by evaporation in a vacuum desiccator. The exact DPN concentration was determined by mixing 0.91 ml of the pooled and concentrated fractions, 0.05 ml of 4 M ethanol in 0.8 M Tris, and 0.04 ml of a 0.1% solution of alcohol dehydrogenase and allowing the mixture to stand at room temperature 30 min. Control reactions where the enzyme or sample was omitted were used as blanks. The absorbances of the solutions were then determined. The absorbance maximum at 340 nm which is characteristic of DPNH (E = 6220) was used to calculate the concentrations of DPN. Aliquots of the sample solution were also spotted on Millipore filters, dried, and counted in a Beckman liquid scintillation counter. The specific activity of the DPN solution was calculated and compared to the specific activity of the original [^{14}C]niacin used.

Assay for Nicotinamide Deamidase—The reaction mixture for enzyme activity determination consisted of 3 μl of 3.8 mM [^{14}C]-niacinamide (specific activity = 13.2 mCi per mmole) and 100 μl of enzyme solution (supernatant or cell extract as the case may be, see the legend to Table II). The mixture was incubated at 37° and stopped by heating in a 100° water bath for 2 to 3 min. The reaction products were assayed by paper electrophoresis which was performed in a Beckman paper electrophoresis cell (Durrum type) with the system in equilibrium with 0.02 M citrate buffer, pH 5.50. Five microliters each of picric acid, 10 mg per ml of niacin, and 10 mg per ml of niacinamide were spotted in the first three strips of paper to serve as markers. On the other strips, 20 μl of each reaction mixture were spotted. Electrophoresis was run for 2 hours, strips were dried in an oven at 100°, and marker spots were visualized under ultraviolet light to determine the location of the niacin and niacinamide segments. Each strip of paper was cut at 1-cm intervals starting from the origin. Radioactivity in each segment was counted by a Beckman LS-100 liquid scintillation counter.

The assay is linear between cell extract concentrations of 85 to 340 μg per ml for incubation times which yield less than 90% conversion of substrate to product.

Other Methods—All other methods used were described in a previous paper (5).

RESULTS

Niacin Uptake as Function of Niacin Concentration—In previous studies with the niacin-requiring *E. coli* strain 15T$^-$ nic$^-$, it was shown that at concentrations of external niacin under 10^{-6} M, the uptake of niacin limits the growth rate of the cell (6). In the experiments described below, the rate of niacin uptake is determined by measuring the intracellular niacin level during balanced growth in media containing different niacin concentrations. Since low niacin concentrations change the generation time of a niacin-requiring strain and may cause undesirable metabolic side effects which could influence the uptake process, we have studied the uptake of niacin in the parent strain 15T$^-$ which is prototrophic for niacin. In this strain, the generation time is unaffected by the extracellular niacin concentration.

Fig. 1 shows a typical experiment from which the intracellular concentration at a particular external concentration of niacin is calculated. The ratio of the number of cells to the intracellular activity can be used to calculate the number of niacin molecules each cell has taken up from the medium. In all cases, the uptake is calculated under conditions wherein the niacin concentration in the medium has not been changed significantly due to growth of the bacteria. The amount of exogenous niacin taken up per cell is plotted as a function of the external niacin concentration on a logarithmic scale in Fig. 2. At low niacin concentrations, there is a linear correlation between niacin taken up and the external concentration. However, at about 10^{-5} M niacin, there is a break in the curve indicating that at higher external concentrations, no further increase in uptake occurs. With a double reciprocal plot, the data for concentrations of external niacin below 5×10^{-6} M fit a straight line which extrapolates to the origin. This indicates that at these concentrations of niacin, the uptake mechanism is far below saturation.

Endogenous Synthesis versus Uptake—We have established the rate of uptake of exogenous niacin as a function of the external niacin concentration. However, these cells have the

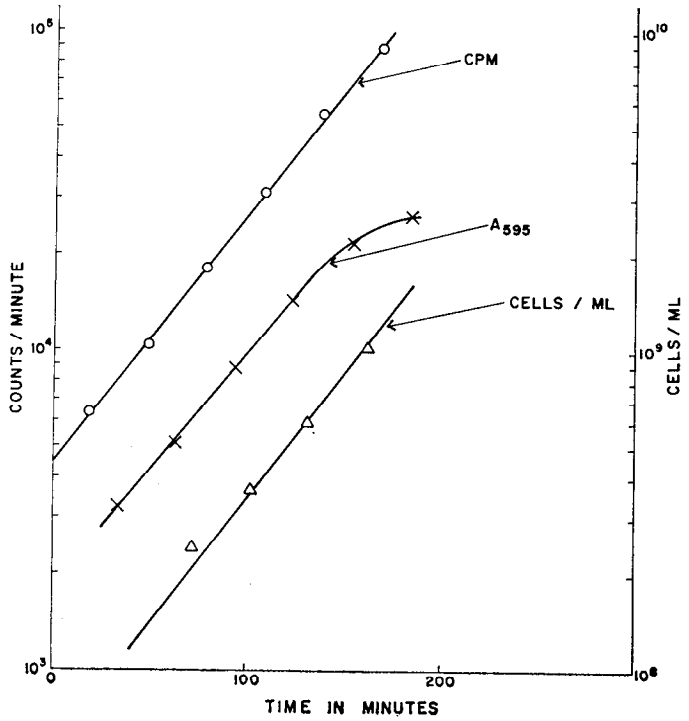

FIG. 1. The determination of niacin uptake by *Escherichia coli* 15T⁻. A culture of *E. coli* 15T⁻ was grown at 37° in an M9 medium supplemented with glucose, thymine, amino acids ("Experimental Procedures"), 1.63×10^{-6} M nicotinic acid (1.16×10^{7} cpm per μmole). At the times indicated, 50-μl aliquots were taken for Coulter counts, 0.5-ml samples for absorbance readings at 595 nm, and 1-ml aliquots for filtering on Millipore filters to measure niacin uptake. The results of these determinations are shown on the figure.

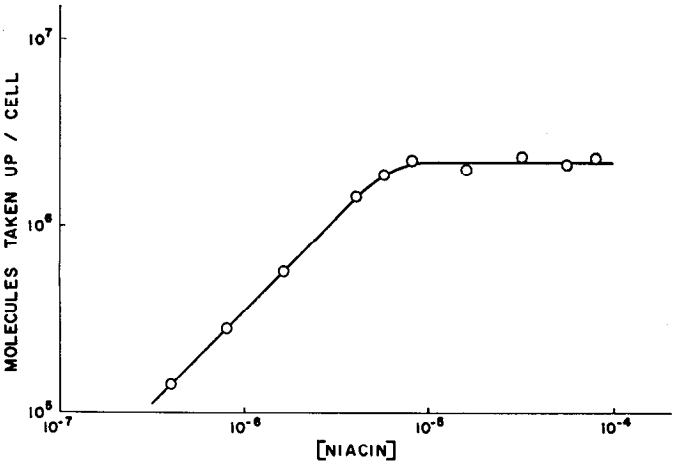

FIG. 2. A logarithmic plot of niacin uptake as a function of the niacin concentration in the medium. The number of niacin molecules taken up per cell was calculated from the amount of radioactivity per cell (a figure derived from experiments of the type described on Fig. 1) and the specific activity of the niacin ("Experimental Procedures"). The data are plotted on a logarithmic scale.

capacity to synthesize the niacin ring endogenously. The dependence of both the endogenous niacin synthesis, and total intracellular niacin levels on external niacin concentration has been investigated.

Bacteria grown in cultures containing different concentrations of [¹⁴C]niacin are collected, lysed, and their DPN is purified by column chromatography. The specific activity of the DPN is then determined ("Experimental Procedures"). From a comparison of the specific activities of the input [¹⁴C]niacin and the purified DPN, the amount of endogenous synthesis can be determined. The data from these experiments are shown on Table I. In Fig. 3, the total cellular niacin and that portion derived from exogenous niacin is plotted as a function of the external niacin concentration.

Endogenous synthesis is completely repressed by an external niacin concentration of 4×10^{-6} M. The total niacin concentration remains fairly constant below external concentrations of 4×10^{-6} M, so that in this concentration range, the amount taken up from the medium is equivalent to the amount of endogenous synthesis suppressed. However, between 4 and 8 μM

TABLE I

Calculation of total intracellular niacin levels at different niacin levels in medium

Cells were grown in different levels of radioactive niacin and the intracellular DPN was isolated as described under "Experimental Procedures." The absorbance at 340 nm of the partially purified DPN was determined after reduction with alcohol dehydrogenase; the same aliquot used to determine absorbance was also used for radioactivity determination.

External niacin concentration	Purified DPN		Specific activity purified DPN	Specific activity medium niacin	Niacin taken up per cell	Total niacin per cell
μM	A_{340} DPNH	cpm/ml × 10^{-4}	cpm/mole × 10^{-12}	cpm/mole × 10^{-12}	10^{6} molecules	
0.41	0.136	6.32	2.89	23.3	0.139	1.12
0.81	0.155	6.45	2.59	11.6	0.315	1.41
1.63	0.143	11.32	4.92	11.6	0.652	1.53
4.06	0.169	12.36	4.55	4.65	1.52	1.54
8.13	0.159	12.73	4.98	4.65	2.50	2.32

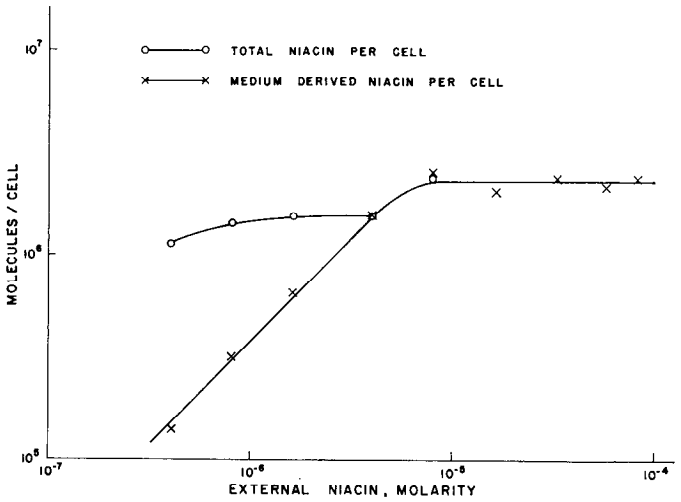

FIG. 3. The relative contribution of exogenous uptake and endogenous synthesis to the total niacin pool as a function of the external niacin concentration. Cells were grown in media containing different concentrations of [¹⁴C]niacin. Cell extracts were prepared and chromatographed on DEAE-Sephadex; the specific activity of the isolated DPN was determined ("Experimental Procedures"). The ratio of the specific activity of [¹⁴C]-nicotinic acid in the growth medium to the specific activity of the isolated DPN was multiplied by the number of medium-derived nicotinic acid molecules per cell at a given external nicotinic acid concentration (see Fig. 2) to give the total nicotinic acid concentration per cell. The points for the medium-derived nicotinic acid per cell at external nicotinic acid concentrations above 10^{-5} M are taken from Fig. 2.

niacin, there is a 50% increase in the total intracellular niacin concentration.

Since a significant increase in the total level of niacin per cell is observed when niacin is provided in the medium at a concentration above 10^{-5} M, the possibility exists that the intracellular distribution of the niacin moiety changes when the total pyridine nucleotide content per cell increases. A chromatographic analysis of the labeled pyridine nucleotide pool of bacteria grown in 0.41 and 81 μM niacin showed that only TPN and DPN had significant amounts of radioactivity, and no apparent difference in the TPN:DPN ratio was detected between the two growth conditions. The intracellular pyridine nucleotide distribution is therefore not greatly perturbed by varying levels of niacin in the medium.

Nicotinamide Uptake—Both exogenous niacin or nicotinamide can be utilized for the synthesis of pyridine nucleotides by *E. coli*. We have investigated whether the uptake of nicotinamide shows the same dependence on external concentrations as the uptake of niacin. We find that nicotinamide behaves very differently from niacin; at all concentrations below 4×10^{-6} which we have examined, nicotinamide is taken up almost instantaneously by an *E. coli* culture. This suggests that the apparent affinity of the cells for nicotinamide is much higher than for niacin. This is shown directly by the double label experiment shown in Fig. 4. In this experiment, [^{3}H]niacin and [^{14}C]nicotinamide at equivalent concentrations (4.1×10^{-7} M) are added to a culture of *E. coli* 15T$^-$. Over 80% of the [^{14}C]-nicotinamide is taken up before uptake of any significant amount of [^{3}H]niacin.

Because of the high apparent affinity of these cells for nicotinamide, we have attempted to clarify whether the cells can concentrate nicotinamide. Nicotinamide deamidase is the only enzyme which is known to act on nicotinamide in *E. coli*. If a deamidase negative mutant were grown in the presence of nicotinamide, one would expect that the free vitamin should accumulate inside the cell if an efficient permease for nicotinamide were present. We have tested such a nicotinamide deamidase$^-$ mutant and our results indicate that this mutant is totally unable to concentrate this compound. Thus, the enzyme nicotinamide deamidase appears to be essential for the permanent retention of the nicotinamide moiety within the cell.

E. coli growing in a medium containing nicotinamide can be shown to convert a fraction of the nicotinamide to extracellular nicotinic acid. As shown in Fig. 5, the extracellular nicotinamide is completely converted to nicotinic acid at low concentrations of medium nicotinamide, and partially converted to nicotinic acid at high concentrations of medium nicotinamide. Since these results raise the possibility that the enzyme which hydrolyzes nicotinamide to nicotinic acid is excreted into the medium, we assayed for nicotinamide deamidase activity in the culture medium (Table II). All enzymatic activity was associated with cells and there was no detectable extracellular enzyme. Experiments using the osmotic shock treatment developed by Anraku and Heppel (10) showed that the enzyme was not present in the periplasmic space between the outer and inner cell membrane.

Assays were also done to determine whether the levels of nicotinamide deamidase varied with the growth medium. Enzyme assays on cells grown at several concentrations of nicotinic acid and nicotinamide in the medium yielded constant specific activities for all growth conditions (~4 units per g). We conclude that this enzyme is constitutive.

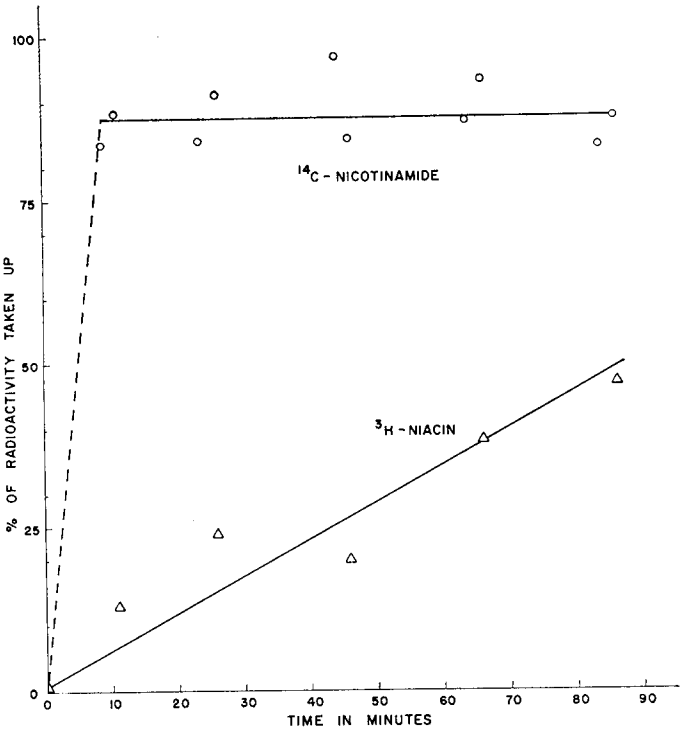

FIG. 4. A comparison of the uptake of niacin and nicotinamide by *Escherichia coli* 15T$^-$. A culture of *E. coli* 15T$^-$ was grown in M9 medium containing no niacin or nicotinamide at 37° with vigorous aeration. At 170 min after the time of inoculation, [^{3}H]nicotinic acid and [^{14}C]nicotinamide were added, each at a concentration of 0.41 μM (the specific activity of [^{14}C]nicotinamide was 5 mCi per mmole, and of [^{3}H]niacin was 787 mCi per mmole). Two-milliliter aliquots were taken at various times and filtered on Millipore filters, the filters being washed before and after the sample was applied with M9 medium supplemented with 40 μg per ml of nicotinic acid and 40 μg per ml of nicotinamide. The amount of radioactivity taken up from the medium at any given time was also determined by counting the radioactivity of 0.2-ml samples from the growth medium at various times: samples were centrifuged in an SS 34 rotor, Sorvall RC2B centrifuge at 7K for 5 min to pellet the cells and the radioactivity in 0.2 ml of supernatant determined.

DISCUSSION

This paper deals with alternative precursors for the nicotinamide moiety in pyridine nucleotides. The pyridine ring may either be synthesized endogenously, or may be derived from exogenous nicotinamide or nicotinic acid.

The endogenous biosynthetic route is least preferred. At concentrations in the culture medium between 0.8 and 4 μM nicotinic acid the increase in uptake of nicotinic acid is compensated for by a corresponding decrease in the rate of endogenous synthesis. Thus, at an external niacin concentration of 8×10^{-7} M, an average cell is taking up 90 molecules per s from the medium and synthesizing *de novo* 340 molecules per s; at 2×10^{-6} M, 215 molecules per s are derived from the medium and 215 molecules per s are being synthesized by the cell. At 4×10^{-6} M, endogenous synthesis of the niacin molecules is completely suppressed and all the intracellular niacin is being derived from the external medium. The results of Chandler *et al.* (4) with a cell-free system are consistent with these *in vivo* observations. They find that the *de novo* synthesis of nicotinic acid by extracts is considerably decreased in cells which have been grown in the presence of nicotinic acid. Our studies define at what concentrations of niacin in the medium this suppression is manifested (11).

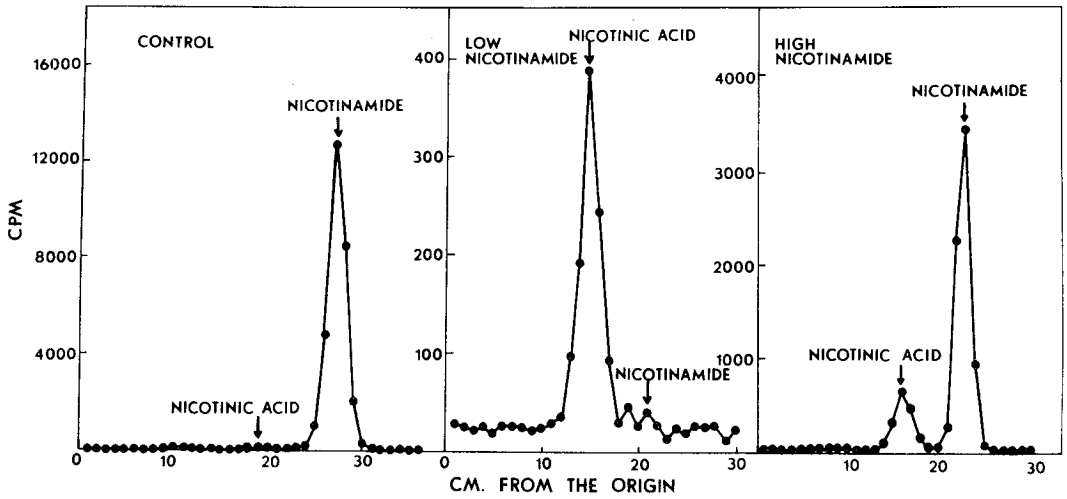

FIG. 5. Conversion of nicotinamide to extracellular nicotinic acid by *Escherichia coli*. Cultures of *E. coli* 15T⁻ nic⁻ (prepared by inoculating 10 ml of medium with 0.2 ml of an overnight culture) were grown in media containing low (0.05 μg per ml) and high (0.5 μg per ml) [^{14}C]nicotinamide (13.2 mCi per mmole). After 5½ hours of growth, the cells were sedimented out of the medium, and the supernatant solutions (0.3 ml of the high and 0.6 ml of the low nicotinamide media) were analyzed by chromatography on DEAE-paper, with carrier nicotinic acid and nicotinamide added as visual markers. As a control, the [^{14}C]-nicotinamide used in these experiments was also run on the chromatogram.

TABLE II

Conversion of nicotinamide to nicotinic acid by extracts of Escherichia coli 15T⁻ nic⁻ and by culture medium

A 10-ml culture of *E. coli* 15T⁻ nic⁻ was grown in M9 medium for 4 hours. The cells were sedimented and the supernatant saved ("cell-free medium"). The cells were resuspended in 10 ml of M9 medium and disrupted by sonic oscillation ("cell extract"). A 0.1-ml aliquot of both the medium and the extract was used to assay nicotinamide deamidase activity as described under "Experimental Procedures."

Source of enzyme	Incubation time	Conversion to nicotinic acid
	min	%
Cell extract	0	<0.5
Cell extract	60	60
Cell extract	120	98
Cell-free medium	120	<0.5

The maximal uptake of exogenous niacin is also tightly regulated. At concentrations of nicotinic acid below 4×10^{-6} M, the amount of exogenous nicotinic acid taken up per cell is a linear function of the external concentration. Under the growth conditions used, with a generation time of 2400 s, this may be expressed as: $R = 1.10 \times 10^{8} \times (N)$ where R is expressed in molecules taken up from the medium per cell in 1 s; and (N) is the molar concentration of external nicotinic acid. However, at concentrations of 10^{-5} M niacin and above, a further increase in the external concentration of nicotinic acid does not result in additional uptake. The maximal rate of niacin uptake appears to be 630 molecules per cell per s.

The results of Imsande and Pardee (3) provide a biochemical basis for the latter observations. They found that cells grown in the presence of high levels of nicotinic acid synthesized far less N_aMN pyrophosphorylase than did cells grown in the absence of nicotinic acid, and that no other enzymes in the Preiss-Handler pathway were similarly affected. The regulation of N_aMN pyrophosphorylase is sufficient to explain control of the nicotinic acid pathway at concentrations of niacin $\geq 10^{-5}$ in the medium.

We have also studied nicotinamide as a precursor for pyridine nucleotide. At low concentrations, our data indicate that the nicotinamide uptake rate is greater than the niacin uptake rate. We have also shown that nicotinamide in the growth medium is converted to extracellular nicotinic acid.

Why is nicotinamide taken up by the cell more rapidly than nicotinic acid? We cannot distinguish between two possible alternatives: nicotinamide deamidase may facilitate entry of nicotinamide into the cell, or alternatively, the near neutral nicotinamide moiety may be able to penetrate the membrane more rapidly than the charged nicotinic acid molecule. Both of these explanations would be consistent with the finding that nicotinamide deamidase mutants do not accumulate nicotinamide in any form, since the latter hypothesis predicts that any intracellular nicotinamide would be rapidly washed out when the cells are harvested. Since nicotinamide deamidase⁻ mutants excrete nicotinamide from the cell quite efficiently, we tend to favor the hypothesis that nicotinamide can go in and out of the cell relatively rapidly compared to nicotinic acid. This is supported by the observation that the more highly charged quinolinic acid is an even less efficient precursor for pyridine nucleotide than is nicotinic acid.[1]

The fact that substantial pools of niacin or nicotinamide are never found within the cell suggests that transport of nicotinamide and niacin against a concentration gradient does not take place. The relative insensitivity of niacin uptake to cyanide is also consistent with the lack of active transport (11).

We can therefore summarize the picture obtained for the accumulation of the nicotinamide moiety in the pyridine nucleotides. There is an endogenous pathway which is operative in *E. coli*; this pathway is inhibited if any exogenous source of the nicotinamide moiety is available. Exogenous niacin and nicotinamide probably penetrate the membrane without requiring any special energy-consuming permeases. Niacin is a charged molecule and, therefore, appears to have some difficulty penetrating the membrane. At low concentrations ($<8 \times 10^{-6}$ M) the rate of penetration of nicotinic acid appears to be rate-limiting in the over-all uptake process; however, at higher concentrations of niacin, more than enough molecules enter the cell,

[1] R. K. Gholson, personal communication.

and when this occurs, the rate-limiting process is the N_aMN pyrophosphorylase reaction.

With nicotinamide, there is apparently no penetration problem and the amide enters the cell rapidly. A constitutive enzyme, nicotinamide deamidase, converts the nicotinamide to nicotinic acid, and if more is converted to nicotinic acid than can be used by the cell for pyridine nucleotide synthesis, then the excess nicotinic acid diffuses back into the medium. It has been postulated that intestinal microorganisms play a role in the deamidation of nicotinamide to nicotinic acid in mammals (2, 7). The results above provide a mechanism for this conversion by the bacterium *E. coli*.

Acknowledgments—We are indebted to Doctors R. K. Gholson and Jerry Chandler for their generous gift of niacin-requiring strains. We would like to thank Dr. Lourdes J. Cruz for helpful discussions.

REFERENCES

1. Ngo, D. T. C. (1971) *J. Manila Med. Soc.* **9,** 47
2. Chaykin, S. (1967) *Annu. Rev. Biochem.* **36,** 149
3. Imsande, J., and Pardee, A. B. (1962) *J. Biol. Chem.* **237,** 1305
4. Chandler, J. L. R., Ogasawara, N., Gholson, R. K., and Andreoli, A. J. (1966) *Fed. Proc.* **25,** 217
5. Lundquist, R., and Olivera, B. M. (1971) *J. Biol. Chem.* **246,** 1107
6. Lundquist, R., and Olivera, B. M. (1973) *J. Biol. Chem.* **248,** 5137
7. Ijichi, H., Ichiyama, A., and Hayaishi, O. (1966) *J. Biol. Chem.* **241,** 3701
8. Gholson, R. K., Tritz, G. J., Matney, T. S., and Andreoli, A. J. (1969) *J. Bacteriol.* **99,** 895
9. Lang, L. (ed) (1961) *Absorption Spectra in the Ultraviolet and Visible Region* Vol. III p. 277, Academic Press, New York
10. Anraku, Y., and Heppel, L. A. (1967) *J. Biol. Chem.* **242,** 2561
11. Olivera, B. M., and Lundquist, R. (1971) *J. Mol. Biol.* **57,** 263